Method for detecting the effect of different chemotherapeutic agents and/or radiation therapy in malignant diseases and method for selecting more effective therapeutic agents for the therapy thereof

Background of Invention

The invention relates to a method for detecting the effect of different chemotherapeutic agents and/or radiation therapy in malignant diseases, wherein the expression profile of tumor and/or cell growth and/or apoptosis-associated genes and/or individual differences (mutations) in gene sequences are determined. Changes associated with chemotherapeutic agents and/or radiation therapy are identified, represented and diagnostically evaluated. The invention further relates to a method for selecting more effective therapeutic agents for the therapy of malignant diseases. The status of cell cycle genes and/or apoptosis-associated target genes or gene products thereof in body fluids, cells and/or organs is determined and diagnostically evaluated to determine their effect on corresponding therapeutic agents. In a preferred embodiment, Bax and p53 expressions or mutations are investigated and the findings therefrom are used for deciding individual-specific therapy in leukemia and other malignant diseases.

The process of a malignant change of a cell begins very early, often with only a single change in the genetic material. It goes through different stages until it produces the degenerated cell and is not yet complete even at this stage.

Progress in modern molecular biology, accompanied by a better understanding of the development of malignant changes on the molecular level, has yielded a multitude of new types of information on the factors involved in the process of carcinogenesis and tumor progression. However, these findings also clearly show how varied, different and complex changes in the molecular network are in order to ultimately be manifested as

a malignant phenotype or therapy-resistant tumor. The involvement of various factors in the process of tumor development is, on the one hand, an indication of the complexity of such a process, but they can also be utilized for diagnostic purposes as well as for a prognostic risk assessment.

In spite of the state of knowledge which has been achieved until now, the staging and the treatment of tumor patients based on it continues to be carried out according to histopathological or clinical criteria. This conventional classification is therefore still decisive for making all therapeutic decisions (Hämatologie Onkologie, ed. P.C. Ostendorfund S. Seeber, Urban and Schwarzenberg 1997; Kompendium Internistische Onkologie, ed. H.J. Schmoll, K. Höffken, K. Possinger, Springer 1997).

Until now, an individual-specific treatment based on a maximally extensive molecular characterization of the tumor could not be carried out.

SUMMARY OF THE INVENTION

Therefore, the purpose of the invention is to use findings on the molecular level for an individual-specific tumor therapy and for finding an effective selection of therapeutic agents in other to provide affected patients with an efficacious treatment.

Tumorigenesis, tumor progression and resistance to therapy are determined by cell cycle and apoptosis-regulating factors. The present invention was based on the unexpected finding that by determining their expression profile, one can use these tumor or cell growth-associated genes as prognostic markers and can correlate them with the response of the patient to different chemotherapeutic agents, radiation therapy and clinical parameters. Based on the obtained marker profile, an effective and promising form of therapy can then be derived for the patients.

The invention therefore relates to a method for detecting the effect of different chemotherapeutic agents and/or radiation therapy in malignant diseases. In accordance with the invention, the expression profiles of tumor and/or cell growth-associated genes and/or individual differences (mutations) in the gene sequences are determined and interactions (associations) with chemotherapeutic agents and/or radiation therapy are identified, represented and diagnostically evaluated.

It has been established that the disturbance of apoptosis together with the deregulation of the cell cycle is a decisive mechanism with regard to the development, growth and progression of malignant tumors. The restoration of the capability of cell cycle arrest and of apoptosis-promoting genes is already an important goal of experimental gene therapeutic strategies. It was found than an inhibition of apoptosis signal cascades can lead to resistance against cytostatic and radiation therapy, which is of enormous clinical importance above all with solid tumors. These resistance mechanisms can originate from disturbances of apoptosis and the cell cycle in that pathologically relevant changes are contained in specific genes and their gene products. Important tumor genes which are responsible for resistance mechanisms are, for example, the genes of the Bcl-2 family, mainly Bax, p53, p16, caspases, Rb, cyclins, inhibitors of cyclin-dependent kinases (CDKIs), ATM and inhibitors of apoptosis proteins (IAPs).

In accordance with the invention, it is preferable to determine the expression profiles and/or mutations of the above-mentioned genes by means of protein or DNA/RNA analyses. During this process, the expression profiles and/or mutations of the respective individual genes are evaluated. But the profiles and/or mutations of different genes can also be combined, whereby the drawing up of individual schemes of treatment is improved and an individual prognosis and risk assessment is possible. Preferably, expression profiles of Bax, p53, p16, caspase and/or

Rb genes or their mutations will be used and diagnostically evaluated. It is especially preferable to identify the status of p53 genes and Bax genes or of their gene products.

This invention particularly concerns a method for selecting more effective therapeutic agents for the treatment of malignant diseases. It is characterized by the determination of the status of cell cycle genes and/or apoptosis-associated target genes or their gene products ex vivo in body fluids, cells and organs, which is diagnostically evaluated in connection with the effect of corresponding therapeutic agents.

Therapeutic agents in accordance with the invention are known agents for the treatment of leukemic or lymphoma diseases and of other malignant diseases like, for example, tumors of the gastrointestinal tract, pancreas, prostate, gynecological tumors (e.g. ovary, cervix, breast), sarcomas, brain tumors, skin and lung tumors as well as tumors of endocrine organs like, for example, the thyroid etc.

These include the well-known cytostatic agents, mainly steroid hormones (e.g. prednisone, prednisolone, methylprednisolone, and other glucocorticoids), antimetabolites (cladribine (2-CDA), fludarabine, mercaptopurine, arabinoside C, 5-substituted dideoxynucleosides, like 5-fluoruracil, azidothymidine), alkylating agents (e.g. mafosfamide, chlorambucil, melphalan, cyclophosphamide), taxanes (like paclitaxel, docetaxel), anthracyclines (e.g. idarubicin, doxorubicin, epirubicin, mitoxanthrones), topoisomerase inhibitors (like etoposide), Vinca alkaloids, (vincristine, vinblastine, vinorelbine), cisplatin and other platinum analogues and many more as well as radiation therapy.

The invention will be explained in more detail below using the example of leukemic diseases. For example, it was established that particularly for the therapy of chronic lymphocytic leukemia

(CLL), the analysis of the Bax expression or mutations showed that with loss of Bax expression a treatment with alkylating agents, anthracyclines and Vinca alkaloids is far less effective. Therefore, such a therapy should be avoided and other forms of therapy should be preferred. The data on which the invention are based clearly show that in patients with CLL and a low Bax expression, the response to therapy with cytostatic agents in vitro is very poor compared with the alkylating agents, mafosfamide, chlorambucil, and melphalan, the anthracyclines, doxorubicin and epirubicin, and the Vinca alkaloid, vincristine. This in vitro sensitivity data correlate with the response to therapy in vivo.

On the other hand, it could be shown that for the treatment of leukemic diseases, chiefly CLL, with low Bax expression, an in vitro therapy with steroid hormones or fludarabine is promising. There is in fact no influence on the responsiveness to steroid hormones (e.g. prednisolone and methylprednisolone) or to fludarabine.

This in vitro data on the chemosensitivity of CLL cells show a good correlation with the clinical response of the patients to the substances in vivo.
Furthermore, they also provide evidence that the diagnostic clarification of the Bax status is essential for making a therapy-related decision. Since the loss of Bax expression can take place at various stages of the realization of genetic information, various detection methods must be included in such a diagnostic procedure. This applies, for example, to the detection of mutative changes on the DNA level (e.g. point mutations, frame-shift mutations) and the detection of hypermethylation patterns within the Bax promoter region as a possible cause for a transcriptional silencing and tests of the expression or expression level of the Bax protein (immunohistochemistry or Western blot or flow cytometry or other quantitative detection

methods) as well as the analysis of transcriptional and translational regulators and the protein breakdown.

The evaluation of the p53 expression or of mutations of the p53 gene has shown that for the treatment of leukemic diseases, particularly of CLL, a therapy with DNA-damaging substances, especially with alkylating agents, anthracyclines and fludarabine, should be avoided if mutations are present within the coding sequence regions of the p53 gene. With regard to a positive correlation of mutations of the tumor suppressor protein p53 and a differential response to cytostatic agents, it could be shown that for patients with mutations within the coded sequence regions of the p53 gene, the response is significantly poorer to fludarabine and DNA-damaging substances like, for example, the alkylating agents. Therefore, for the candidate gene p53 as well, an extensive diagnostic analysis must be carried out on the DNA and protein level. In principle, that also applies for the p53-homologous candidate genes p73 and p41.

Ultimately, via the combined diagnosis of the two candidate genes p53 and Bax, an individualized form of therapy can be applied by drawing up individual schemes of treatment based on the combination of the genetic status of the p53 and Bax genes or of their gene products and/or mutations.

Furthermore, the invention relates to the use of status determination of cell cycle genes and/or apoptosis-associated target genes or their gene products or mutations using protein or DNA/RNA analysis for the determination of the resistance to therapy and for the specific selection of therapeutic agents for cytotoxic therapies. Preferably, the analysis is carried out based on Bax expression or mutations or based on p53 expression or mutations.

Special priority is given to the use of status determination of Bax and p53 genes for a risk-adapted tumor therapy with leukemic

diseases like CLL and other tumors. In another concrete form of the invention, the use occurs in combination of p53 and Bax with other cell cycle and apoptosis regulators, which are also employed either alone or in combination as a molecular pathway (signal pathway) diagnosis with malignant tumors or precancers.

Although it is known that due, for example, to an existing clear functionality of the tumor suppressor protein p53 within the cell cycle and apoptosis regulation, a loss of p53 function is often found in tumors (e.g. via mutation, deletion or promoter changes). It was surprising, however, that a functionally damaged p53 protein nevertheless facilitates a selective response to certain cytostatic agents in patients with chronic lymphocytic leukemia (CLL). That could be proven for the first time. In addition, this also concerns a multitude of other apoptosis-associated target genes and especially their functional combination.

The present investigations were carried out as an example for the tumor suppressor protein p53 and the proapoptotic gene Bax in patients with chronic lymphocytic leukemia (CLL). Additional data are also available on, for example, these and other apoptosis and cell cycle regulators with tumors of the gastrointestinal tract like, for example, gastric carcinoma, esophageal carcinoma and sarcomas.

With the present invention, conclusive evidence could be furnished based on the two apoptosis-influencing proteins p53 and Bax that via a diagnostic characterization of relevant tumor genes on the molecular level (DNA) and on the expression level (protein) that the possibility exists of selectively choosing cytostatic agents in order to achieve an improved treatment. The findings on molecular pathogenesis and resistance to therapy of tumors can, in accordance with the invention, be used as a basis for an individual-specific tumor therapy and, in this way, result

in a more specific and ultimately also maximally successful treatment for the affected patients.

Thus, one can spare patients who have, according to molecular standards, a good prognosis of responding to the cytotoxic therapies and showing improved survival after therapy elaborate and cost-intensive treatments. At the same time, more aggressive treatments can be carried out in patients with a poorer risk profile in order to improve the therapeutic effect. Furthermore, with detected resistance, a less aggressive treatment can be carried out to avoid unnecessary costs and discomfort for patients.

By means of the invention, it is possible to use changed cellular tumor markers as a decision-making criterion for the selection of various standard chemotherapeutic agents; this is, to also take advantage of positive correlations between changed tumor markers and the efficacy or inefficacy of chemotherapeutic agents. This offers the possibility, after a characterization of selected cellular tumor markers, to selectively chose the form of the chemotherapeutic agent, radiation therapy, or their combination which is to be employed.

The following table 1 shows the effect of various cytostatic agents for a CLL treatment. It is based on comparative studies of the expression of Bax protein, which is known to be proapoptotic with CLL, and the Bcl-2 protein, which functions to block cell death. It is clear from the table that the response to anthracyclines and alkylating agents (doxorubicin, epirubicin, chlorambucil and mafosfamide) as well as vincristine is highly correlated with the level of Bax expression (Western blot) (p-value significant and < 0.05). In contrast, no correlation was found with determination of the Bcl-2 expression. On the other hand, the effect of the steroid hormones (methylprednisolone, prednisolone) and of fludarabine administration showed no correlation to the level of the Bax expression, which indicates

that the agents have an increased cytotoxic effect even on Bax-negative tumor cells.

These conclusions are also confirmed by the correlation of Bax and the ex-vivo response of CLL patients to a number of cytostatic agents which is shown in Figure 1. The figure gives the relative level of Bax protein expression (densitometric values from Western blot analyses) and the LC90 dose for each of the cytostatic agents.

Figure 2 shows the correlation between Bax expression and the LC90 dose of doxorubicin in 37 CLL patients.

Figure 3 shows the reduced sensitivity of CLL cells to cytostatic agents in vitro versus the alkylating agents chlorambucil and melphalan as well as versus fludarabine for p53-mutant CLL patients compared to the p53 wild type. The p53 mutations were determined for the exons 5 to 8 using SSCP-PCR. The height of the bars corresponds to the dose of the cytostatic in μg/ml.

Table 1:
Relationship between the level of the protein expression of Bax, Bcl-2, and the ratio of Bax and Bcl-2 expression (Bcl-2/Bax) and the ex-vivo cytotoxicity of cytostatic agents in the treatment of CLL.

Significance level of the Pearson correlation coefficient (p-value)			
Cytostatic agent	**Bax**	**Bcl-2**	**Bcl-2/Bax**
Fludarabine phosphate	0.816	0.212	0.829
Cladribine (2-CDA)	0.524	0.351	0.658
Chlorambucil	0.034	0.739	0.157
Mafosfamide	0.017	0.250	0.160
Methylprednisolone	0.836	0.415	0.880
Prednisolone	0.807	0.545	0.898
Vincristine	0.011	0.052	0.127
Doxorubicin	0.001	0.920	0.001
Epirubicin	0.002	0.647	0.001

P-values smaller than 0.05 are statistically significant

Figure captions:

Figure 1:
Association of the level of Bax and the ex-vivo response of CLL patients to a number of cytostatic agents. The figure shows the relative level of Bax protein expression (densitometric values from Western blot analyses) and the LC90 dose for each of the cytostatic agents.

Figure 2:
Association between Bax expression and the LC90 dose of doxorubicin in 37 CLL patients. This relationship with regard to the cytotoxic effect of the cytostatic agent could only be observed for Bax, but not for Bcl-2 or the ratio of Bcl-2 to Bax.

Figure 3:
Reduced sensitivity to cytostatic agents (height of the bars corresponds to the dose of the cytostatic in µg/ml) for p53-mutant CLL patients compared to the p53 wild type. The p53 mutations were determined for the exons 5 to 8 using SSCP-PCR.